A novel immune evasion mechanism of LMP-1, an EBV-primary oncogene, in nasopharyngeal Carcinoma

A novel immune evasion mechanism of LMP-1, an EBV-primary oncogene, in nasopharyngeal carcinoma.

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Abstract

Nasopharyngeal carcinoma is an Epstein-Barr virus (EBV)-associated tumor. Viruses that are associated with malignant transformation have evolved unique mechanisms to interfere with this interaction to evade antiviral T-cell responses. EBV exploits many immune evasive strategies to successfully establish a latent infection in B cells. $CD8^+$ T-cell responses to LMP-1 are generally very low and rarely detected in healthy virus carriers. Activation of the NF-kB pathway by EBV–LMP-1 leads to an up-regulation of the MHC class I antigen–processing pathway. Paradoxically, LMP-1 itself induces a subdominant $CD8^+$ T-cell response and appears to have evolved to avoid immune recognition. An expression of LMP-1 in human cells enhanced the *trans*-presentation of $CD8^+$ T-cell epitopes, however, *cis*-presentation of LMP-1–derived epitopes was severely impaired. Deletion of the first transmembrane domain of LMP-1, which prevented self-aggregation, significantly enhanced *cis*-presentation of T-cell epitopes from this protein, whereas it lost its ability to up-regulate *trans*-presentation. These results delineate a novel mechanism of immune evasion, which renders a virally

encoded oncogene inaccessible to the conventional MHC class I pathway limiting its *cis*-presentation.

Epstein-Barr virus (EBV) exploits many immune evasive strategies to successfully establish a latent infection in B cells and epithelial malignant tumors such as nasopharyngeal carcinoma (NPC). Analysis of the role of individual EBV latent antigens in the regulation of antigen-processing genes indicated that latent membrane protein-1 (LMP-1) was sufficient to up-regulate expression of transporters associated with antigen processing and *trans*-presentation of MHC class I–restricted epitopes in B cells1). Subsequent studies demonstrated that the immunomodulatory effects of LMP-1 are mediated through C-terminal activator regions (CTAR1 and CTAR2), which are involved in the induction of the nuclear factor–κB (NF-κB). At the same time, LMP-1 promotes invasion and metastasis through these intracellular activation domains2-4). Paradoxically, the population of $CD8^+$ T-cell that responses to LMP-1 is generally very low and rarely detected in healthy virus carriers, suggesting that LMP-1 may limit its

cis-presentation through the MHC class I pathway. We have delineated a mechanism by which LMP-1 limits its self-presentation without compromising its ability to modulate *trans*-presentation of $CD8^+$ T-cell epitope5, 6)

To assess *trans*-presentation of LMP-1, we transfected multiple LMP-1 sequences to epithelial cell lines. Although all LMP-1 variants up-regulated the expression of MHC class I on a human epithelial cell line, C33A, considerable activation of LMP-1-specific T cells was only evident after incubation with peptide-sensitized cells. In contrast, LMP-1 expression in C33A cells enhanced trans-presentation of $CD8^+$ T-cell epitopes from another EBV-encoded membrane protein, LMP-2A. Collectively, these results suggested that, despite its capacity to up-regulate *trans*-presentation via the MHC class I pathway, LMP-1 limits its self-presentation to $CD8^+$ T cells.

Much of the data presented are based on either stable or transient expression of LMP-1. To confirm these observations, we assessed endogenous presentation of LMP-1 epitopes in EBV-transformed LCLs. Although activation of LMP-2A specific T cells was evident after incubation with HLA-matched LCLs, very low levels of activation of T cells

specific for multiple LMP-1–encoded peptide epitopes was observed. Activation of LMP-1–specific T cells could be detected after incubation with peptide-sensitized LCLs. To investigate the mechanism for this immune evasion, we tested a series of LMP-1 expression vectors with mutations in the CTAR1 and/or CTAR2 domain; and a deletion mutant commencing at the second methionine of LMP-1 (Δ1-43LMP-1-GFP), which removes the first trans-membrane domain. This domain has been shown to have immunomodulatory effect, andΔ1-43LMP-1 has been used for expanding LMP-1-specific T cells. Although mutations within the CTAR domains had no impact on the presentation of LMP-1 epitopes, deletion of the first *trans*-membrane domain enhanced endogenous presentation of HLA class I–restricted epitopes. Consistent with previous studies, intracellular localization analysis using fluorescent microscopy revealed that, although full-length LMP-1 and its CTAR mutants formed large aggregates in the perinuclear region, Δ1-43LMP-1-GFP lost its ability to aggregate. This enhanced presentation was not the result of increased expression ofΔ1-43LMP-1-GFP compared with full-length LMP-1. These observations indicate that, although the NF-κB signaling domains had no impact on *cis*-presentation of LMP-1 epitopes, aggregation of

this protein (a critical requirement for *trans*-presentation) limits its accessibility to the MHC class I processing machinery.

The data presented in this meeting indicate that, in addition to constraining its *cis*-presentation through aggregation, epitopes encoded within LMP-1 protein are most probably destroyed by cellular proteasomes, providing a dual strategy by which LMP-1 limits self-presentation to $CD8^+$ T cells7).

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